

**Please replace the paragraph at Page 24, lines 25-28, with the following:**

To prepare the frozen dilution cultures for lysis and concentration, the tubes were placed into a 95 °C heat block for five minutes, then put in -80 °C for 15 minutes. This process, which helps insure cell lysis, was repeated once, and the tubes finally thawed at room temperature.

*In the Claims:*

*Please amend the claims to read as follows:*

1. (Amended) A method of isolating and identifying a microbial species from a source environment, comprising:

gathering from the source environment a sample suspected of containing at least one microorganism that has not been cultured using standard culturing techniques;

providing a volume of culture medium to the microorganism in at least one microtiter plate compartment;

incubating the microorganism in the medium for a period of time and in an environment sufficient to result in growth of the microorganism if the medium and environment are capable of supporting such growth to produce a culture sample, wherein growth of the microorganism comprises an increase in the number of microorganisms in the compartment to no more than about  $5 \times 10^4$  cells milliliter;

detecting growth of the microorganism using an automated detection method that comprises removing a portion of the culture sample and depositing the portion onto a surface, wherein growth of the microorganism indicates that the microbial species has been isolated from the source environment; and

identifying the microbial species, wherein identifying the microorganism includes hybridization of a probe to a nucleic acid molecule of the microorganism; amplification of a nucleic acid molecule of the microorganism; immunodetection of a molecule of the microorganism; sequencing of a nucleic acid molecule of the microorganism; or a combination of two or more thereof.

2. (Reiterated) The method of claim 1, wherein a plurality of individual microorganisms are separately incubated in microtiter plate compartments.

3. (Reiterated) The method of claim 2, wherein the plurality is at least 20.
4. (Reiterated) The method of claim 2, wherein the plurality is at least 50.
5. (Reiterated) The method of claim 2, wherein the plurality is at least 100.
6. (Reiterated) The method of claim 2, wherein the plurality is at least 400.
7. (Reiterated) The method of claim 2, wherein the plurality is at least 1000.
8. (Reiterated) The method of claim 2, wherein the plurality is at least 1500.
9. (Cancelled)
10. (Cancelled)
11. (Reiterated) The method of claim 1, wherein the source environment is a non-laboratory environment.
12. (Reiterated) The method of claim 1, wherein the source environment is a natural environment.
13. (Reiterated) The method of claim 1, wherein more than one microorganism is gathered from the source environment.
14. (Reiterated) The method of claim 13, wherein each organism is provided a volume of medium in a separate compartment.
15. (Amended) The method of claim 14, wherein the volume of medium is no greater than about 1 ml.

16. (Reiterated) The method of claim 14, wherein the organisms are placed in the separate compartments using flow cytometry, cell sorting, or dilution.

17. (Amended) The method of claim 1, further comprising counting at least one microorganism that grew.

18. (Amended) The method of claim 1, wherein identifying the microorganism includes hybridization of a probe to a nucleic acid molecule of the microorganism.

19. (Amended) The method of claim 1, wherein identifying the microorganism includes amplification of a nucleic acid molecule of the microorganism.

20. (Amended) The method of claim 1, wherein identifying the microorganism includes immunodetection of a molecule of the microorganism.

21. (Amended) The method of claim 1, wherein identifying the microorganism includes sequencing of a nucleic acid molecule of the microorganism.

22. (Cancelled)

23. (Amended) The method of claim 1, wherein identification of the microorganism is automated.

24. (Reiterated) The method of claim 17, wherein identifying or counting a microorganism comprises depositing cells in a two-dimensional array, such that different cultures arising from different cells each occupy a unique position in the array.

25. (Reiterated) The method of claim 17, wherein identifying or counting a microorganism comprises use of a technique that reveals a genetic or enzymatic property of the microorganisms.

26. (Reiterated) The method of claim 17, wherein a cultured strain of bacteria, called a reporter strain, is added to the medium with an unknown cell from nature, such that production of at least one compound by the unknown cell is revealed by a growth or genetic responses of the reporter strain.

27. (New) The method of claim 1, wherein the detection method comprises removal of substantially all of the medium from the cultured sample.

28. (New) The method of claim 1, wherein identifying the at least one microbial species comprises sequencing a target nucleic acid sequence of the microbial species, and comparing the sequence of the target nucleic acid to at least one known sequence of the target nucleic acid from at least one known organism.

29. (New) The method of claim 28, wherein the target nucleic acid sequence is a ribosomal RNA sequence.

30. (New) A method of isolating and identifying a microbial species from a marine source environment, comprising:

gathering from the source environment a sample suspected of containing a plurality of microorganisms that have not been cultured using standard culturing techniques;

providing a volume of culture medium based on sea water to the plurality of microorganisms in a plurality of microtiter plate compartments, such that each compartment receives no more than about three microorganisms;

incubating the plurality of microorganisms in the medium for a period of time and in an environment sufficient to result in growth of the microorganism if the medium and environment are capable of supporting such growth to produce a plurality of culture samples;

detecting growth of at least one of the plurality of microorganisms using a detect method that comprises depositing a portion of the culture sample onto a surface using a filtration manifold, wherein growth of the microorganism comprises an increase in the number of microorganisms in the compartment to no more than about  $5 \times 10^7$  cells/milliliter, and wherein

growth of the at least one microorganism indicates that the microbial species has been isolated from the source environment; and

identifying the microbial species, using a method that comprises:

sequencing a ribosomal RNA sequence of the microbial species;

comparing the sequence of the ribosomal RNA to at least one known ribosomal RNA sequence from at least one known organism; and

assigning an identity to the microbial species based on sequence similarity to the ribosomal RNA of the known organism.

### REMARKS

By this amendment, an acknowledgment that this invention was made with government support under National Science Foundation Major Research Instrumentation Grant, No. OIA-9977469 is added. In addition, several obvious typographical errors in the specification are corrected. Claims 1, 15, 17-21, and 23 are amended. Claims 9, 10 and 22 are cancelled and new claims 26-30 are added.

Claim 15 is amended to correct an obvious typographical error. Claims 18-21, and 23 are amended to modify their dependency from cancelled claims. Support for the amendments and for new claims 26-30 can be found in the papers filed by the Applicant on November 21, 2000, in parent case 09 675,382.

No new matter is added by these amendments. Unless specifically stated, none of these amendments are intended to limit the scope of any claim. Upon entry of this amendment, **claims 1-8, 11-21, 23-30 will be pending in this application.** Consideration of the application and entry of the above amendments are respectfully requested.